Polarizing CH–π bonds

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Modulating Weak Interactions for Molecular Recognition: A Dynamic Combinatorial Analysis for Assessing the Contribution of Electrostatics to the Stability of CH–π Bonds in Water**

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Abstract: *Electrostatic and charge-transfer contributions to CH–π complexes can be modulated by attaching electron-withdrawing substituents to the carbon atom. While clearly stabilizing in the gas phase, the outcome of this chemical modification in water is more difficult to predict. Herein we provide a definitive and quantitative answer to this question employing a simple strategy based on dynamic combinatorial chemistry.*

Nowadays it has become clear that CH–π interactions[1–3] play a key role in a variety of molecular recognition processes[4,5] including conformation stabilization,[6] crystal packing,[7] formation of gas-phase clusters,[8] or chiral discrimination.[9] Nevertheless, there are still aspects of these interactions that remain poorly understood.

Most theoretical analyses have revealed that, in the gas phase, the stability of CH–π complexes largely arises from dispersion forces.[2,3] However, although relatively small, electrostatic interactions cannot be neglected, because they contribute to both the strength and the directionality of the contact. Actually, this dual dispersive/electrostatic nature represents an essential feature of CH–π bonds and explains their ubiquitous presence under different environments.[2] It is important to note that electrostatic forces can be enhanced by the presence of electron-withdrawing substituents attached to the interacting carbon atom (Figure S1). Indeed, in the gas phase, polarized CH moieties form tighter complexes with aromatic systems than nonpolarized ones.[3] Similarly, replacement of the CH group by an OH function (which, from an electrostatic perspective, might resemble an extremely polarized CH) leads to a larger enhancement in stability.[3] Hence, CH polarization represents a simple chemical strategy for the stabilization of CH–π complexes in the absence of solvent.

The outcome of this simple chemical modification in water is, however, more difficult to predict. In fact, exposed ROH–π contacts[10] are usually highly destabilized in water due to the competence provided by the stronger and ubiquitous ROH···H_2O hydrogen bonds. Analogously, the polarization of a particular CH bond could also enhance its own polar interactions with the solvent, thus leading to a minor stabilization, a null effect, or even a significant destabilization of the CH–π contact (Figure S1). However, to the best of our knowledge, no unambiguous experimental evidences have been presented so far to support or disprove the stabilizing role of CH polarization in water. Moreover, the corresponding effect, if any, has never been quantified.

These questions have profound implications for the molecular recognition and drug design fields, and providing the right answers will contribute to the longstanding challenge of unravelling the complicated influence of water on molecular association.[11]

To this aim, we have employed a modified version of the simple strategy recently developed by us,[12] based on the principles of dynamic combinatorial chemistry.[13] This methodology is schematically represented in Figure 1 (see also Figures S2–S10). First, several model systems, including a reactive 3-amino-α-D-allopyranose scaffold (unit I in Figure 1) and alternative CH–π donor units (dubbed II and IIa in Figure 1) were designed and synthesized. As a next step, buffered water solutions containing equimolecular amounts of two or more specific model compounds were treated with an arylacetaldehyde to form a dynamic mixture of hemiaminals/imines in exchange. It should be noted that, in these transient species, the aromatic system is optimally positioned to establish intramolecular interactions with a single face of the alternative CH–π donor units (a conclusion based on our previous experience with related altrose disaccharides and also supported by molecular dynamics calculations). The resulting energy contributions depend on the chemical nature of the CH–π donor and acceptor units, and render the hemiaminal/imine species nonequivalent in terms of stability. Consequently, they exhibit a distinct equilibrium population. Subsequent chemical reduction of the transient species with

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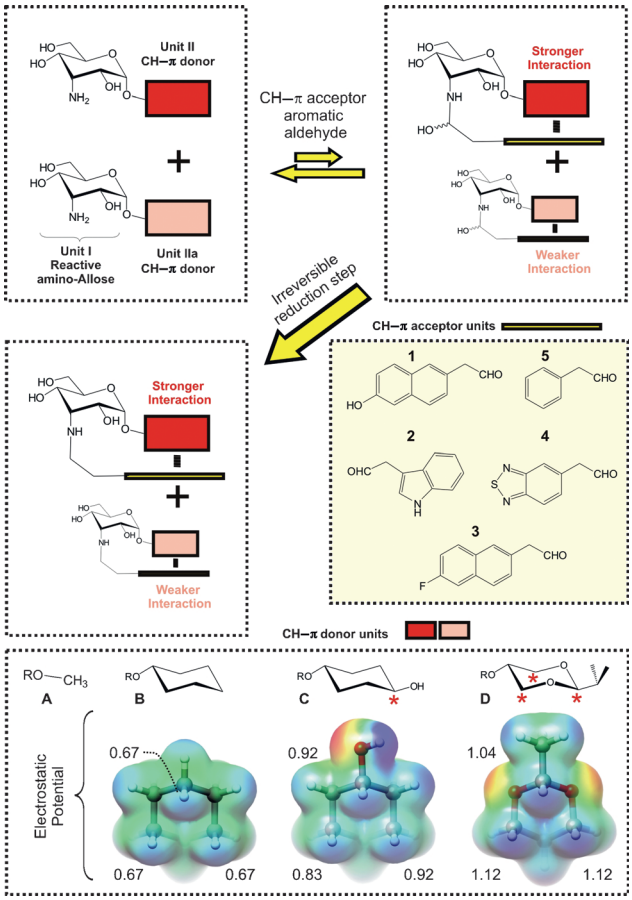

Figure 1. Schematic representation of the dynamic combinatorial approach employed to test the influence of CH polarization on the stability of CH–π complexes (see the main text). Our library of CH–π acceptor systems (**1**–**5**) is represented in the yellow square. The library of CH–π donor units (**A**–**D**) is shown at the bottom together with the calculated electrostatic potential surfaces. Partial charges at the interacting axial hydrogens are also represented.

an externally added reagent irreversibly converts them into a nonequimolecular mixture of secondary amines, whose relative populations can be easily evaluated by NMR methods. These data were then employed to determine the relative interaction free energies (ΔG) of each complex.

The employed library of the novel 3-amino-α-D-allopyranose derivatives (**A**–**D**) and arylacetaldehydes (**1**–**4**) is represented in Figure 1. The former compounds include CH–π donor units with zero (**B**), one (**C**), and three (**D**) polarized CH groups exposed at the interacting surface. Compound **A**, with no CH–π donor unit, was also synthesized as a reference. Regarding the aldehydes, we prepared derivatives equipped with both, electron-rich and electron-poor CH–π acceptor aromatic systems. Compound **5** (2-phenylacetaldehyde) was employed as control, because it incorporates a significantly smaller aromatic ring and is consequently unable to establish optimum contacts with the CH–π donor units upon reaction with the allose scaffold.

We then performed competition experiments in NMR tubes, employing the **A**/**C** pair and the different arylacetaldehydes. Thus, 1:1 **A**/**C** mixtures in buffered water were treated with a substoichiometric amount of the corresponding aldehyde to establish an effective competition (see the Experimental Section). The solution was then allowed to equilibrate and then, sodium cyanoborohydride was added. After completion of the reduction step, the relative fraction of the two secondary amine products was evaluated from the well-resolved NMR signals. The obtained results are represented in Figure 2 and Table 1. With aldehyde **5**, the product ratio (denoted as R in Figure 2) **A**-**5**/**C**-**5** amounts to 1.8–2.0, implying that the interaction energy between the CH–π-

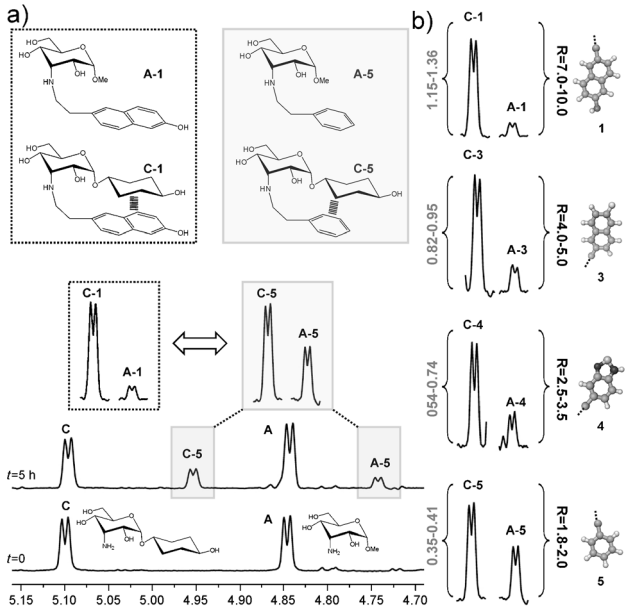

Figure 2. a) Example of competition experiments performed with pair **A**/**C** and aldehyde **5** (see the main text). 1D-NMR spectra (anomeric proton region) collected at $t = 0$ and $t = 5$ h (reaction completion) are shown at the bottom. The ratio between products **C**-**5** and **A**-**5** (represented above) is highlighted (grey-filled square). The outcome of the experiment performed with aldehyde **1** is also represented for comparison (dotted black square). b) Comparison of the product ratios (R, shown in black) obtained with different aldehydes. Interaction free energies (kcal mol^{-1}) for the CH–π contacts established in the respective products are represented in grey.

Table 1: Net interaction free energies (kcal mol^{-1}) for the CH–π complexes established between donors present in **B**–**C** and the aromatic rings of **1**, **3**, and **4**. Stability differences ($\Delta\Delta G$, kcal mol^{-1}) between complexes involving three and zero polarized CH groups are also represented. These values represent a substantial fraction ($\Delta\Delta G$ (%), right column) of the net interaction free energies estimated for **D**.

	B zero polarized CH–π[b]	**C** one polarized CH–π[a]	**D** three polarized CH–π[b]	$\Delta\Delta G$[c] (**D**–**B**)	$\Delta\Delta G$ [%]
1	0.97 ± 0.11	1.25 ± 0.10	1.94 ± 0.11	0.91 ± 0.09	47
3	0.62 ± 0.07	0.88 ± 0.06	1.28 ± 0.13	0.65 ± 0.06	51
4	0.57 ± 0.11	0.64 ± 0.10	0.84 ± 0.11	0.23 ± 0.08	27

[a] Measured from pairwise competition experiments performed with mixtures **A**/**C**. [b] Estimated from column (**C**), considering the stability differences represented in Figure 4 (see the Experimental Section). [c] Estimated from pairwise competition experiments performed with mixtures **D**/**B**.

donor unit present in **C** and the aromatic system is in the range of 0.35–0.41 kcal mol^{-1}. This relatively small value reflects the reduced size of the phenyl ring. In contrast, with aldehyde **1** (with a larger aromatic ring) the **A-1**/**C-1** product ratio increases to 7.0–10.0, which corresponds to a complex stability of 1.15–1.36 kcal mol^{-1}. It is noteworthy to mention that this interaction energy is similar to that reported for comparable CH–π contacts observed in natural systems. For example, carbohydrate/aromatic interactions (ubiquitous in lectin/sugar complexes) typically involve the formation of two to three simultaneous CH–π bonds and contribute to carbohydrate recognition by 1–2 kcal mol^{-1}.[4]

Finally, replacement of the π-rich hydroxynaphthyl system with electron-deficient aromatic rings, as those present in **3** and **4**, leads to a progressive destabilization of the interactions, as reflected by the obtained **A3**/**C3** and **A4**/**C4** ratios (see Figure 2). These experiments are in agreement with previous knowledge on CH–π interactions and support the validity of our strategy.[4b] Similar tests carried out with pairs **A**/**B** and **A**/**D** were inconclusive due to signal overlapping or poor signal-to-noise ratio.

Dynamic combinatorial experiments performed with more complex mixtures provided key evidences for the role played by CH polarization on the stability of CH–π complexes. More specifically, we carried out simultaneous competition experiments with our four allose derivatives (**A**/**B**/**C**/**D** 1:1:1:1), and one single aldehyde. The fraction of the corresponding reaction products in the final mixture was evaluated by 2D-HSQC experiments to alleviate the significant signal-overlapping present in the NMR spectra. The obtained results provide strong evidence for a highly stabilizing influence of CH polarization (Figure 3).

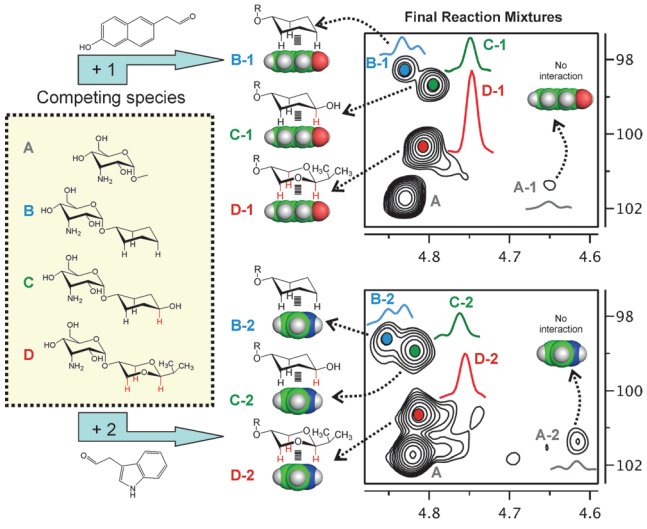

Figure 3. Dynamic combinatorial experiments performed with equimolecular solutions of the four allose derivatives (yellow square) and aldehydes **1** (top) and **2** (bottom). After reaction completion, the final mixtures were analyzed by means of 2D-HSQC experiments (anomeric regions shown). NMR signals of the final products together with the corresponding cross-sections are represented. Their intensities reflect the relative stabilities of the CH–π contacts established in the final products (also shown). NMR signal corresponding to unreacted derivative **A** is also labeled.

Thus, the **B-1**/**C-1**/**D-1** product ratio (with zero, one, and three polarized CH groups involved in contacts with the aromatic ring, respectively) is 1:2:4. Moreover, product **A-1** (with no interaction) is almost undetectable. Analogous experiments performed with aldehyde **2** (Figure 3, bottom) yielded similar results (product ratio **B-2**/**C-2**/**D-2** 1:2:3.7). Although the HSQC provided valuable qualitative information, more precise conclusions were gathered from integration of NMR signals in 1D spectra. Thus, smaller sets of allose derivatives were used for additional competition experiments. The outcome of these experiments is shown in Figure 4. The

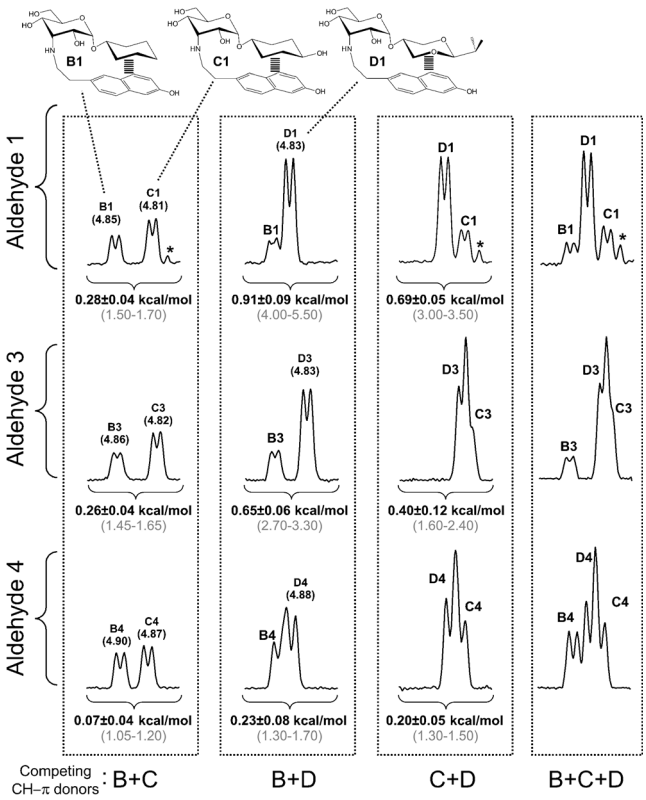

Figure 4. Dynamic combinatorial experiments for the **B**/**C**/**D** triad and **C**/**D**, **B**/**D**, **B**/**C** pairs (from right to left), employing aldehydes **1** (top), **3** (middle panel), and **4** (bottom). NMR signals for the allose anomeric proton in the final products are shown. Chemical shifts (ppm) are indicated above the signals. The measured product ratios (grey), together with the estimated free energy differences between the alternative CH–π complexes (black) are also represented. Overlapping signals were deconvoluted before integration (see the Supporting Information).

net interaction free energies for the alternative CH–π donors, extracted from the results shown in Figures 2 and 4 are collected in Table 1. Interestingly, for electron-rich aromatic rings (such as **1**), the extra stability provided by CH polarization amounts, on average, to 0.3 kcal mol^{-1} per interacting CH group. Accordingly, the complex formed by the 1,3-dioxane unit (with three polarized interacting CH groups) is ca. 0.6 kcal mol^{-1} and 0.9 kcal mol^{-1} more stable than those formed by cyclohexanediol (with one polarized interacting CH group) and cyclohexanol (with no polarized CH groups) rings, respectively (results further confirmed by directly

inspecting the relative population of the intermediate imine/enamines before the reduction step, from concentrated NMR samples Figure S8). Of note, the later value corresponds to a significant fraction (around 50%) of the net interaction energy estimated for the 1,3-dioxane unit. This contribution, together with the global stability of the respective complexes decreases for very electron-poor aromatic rings such as that present in **4**. However, in all cases the stabilization promoted by the attachment of electron-withdrawing groups to the interacting CH functions remains significant (Figure 4 and Table 1).

Finally, addition of organic cosolvents, such as acetonitrile (see Figure S11), leads to a progressive destabilization of all the CH–π complexes, reflecting the attenuation of the hydrophobic effect (i.e., less polar medium) and the appearance of competitive interactions with the more affine alkyl groups of the cosolvent.

In conclusion, CH polarization in water largely stabilizes the CH–π contacts exacerbated by the hydrophobic "solvent cage" effect, proving that electrostatics and charge transfer forces are remarkably relevant. It could be anticipated that more polar CH bonds would also experience stronger interactions with the solvent, and, therefore, larger desolvation penalties. According to our data, even for fully exposed CH moieties as those considered herein, this unfavorable influence does not counteract the improvement in the direct CH–π contacts established upon complex formation.

Finally, from a drug design perspective, we have shown that the attachment of electron-withdrawing atoms to the interacting CH groups is a valid strategy to stabilize CH–π bonds in physiological environments. Larger increases in stability could probably be achieved by employing more electronegative polarizing atoms, such as fluorine.

In our opinion, this work provides an unambiguous and quantitative answer to a simple question that is of general relevance in different fields of chemistry and biology.

Experimental Section

Experimental procedures for the synthesis of derivatives **A**–**D** and **1**–**4** are described in the Supporting Information (SI). Dynamic combinatorial competition experiments were performed as follows. Equimolecular mixtures of two to four derivatives ($\approx$1 mM each) in D_2O (10 mM phosphate, pH 6.2) were treated with a substoichiometric amount of the corresponding arylacetaldehyde. The amount of the later was carefully adjusted to achieve conversions of the allose derivatives not superior to 40% (usually in the 300–600 μM range depending on the number of components present in the mixture). After a 2 h equilibration period, we added sodium cyanoborohydride (5 mM). Formation of the reaction products was followed by 1D-NMR. At reaction completion, qualitative analysis of the final mixtures was performed by looking at the intensity of key signals of the products in 2D-HSQC NMR experiments. We derived the ratio between the alternative secondary amine products by integrating NMR signals in 1D experiments acquired with long relaxation delays. Overlapping signals were deconvoluted before integration employing the line-fitting module implemented in the program MestreNova (see SI). The obtained values were assumed to reflect differences in the population of the corresponding imine/enamine intermediates and therefore, employed to derive the relative stability ($\Delta\Delta G$) of the alternative stacking modes. Our protocol was validated in selected systems by also measuring these values from the relative populations of the intermediate imines/enamines prior to reduction (these species are intrinsically unstable in water but could be detected employing concentrated NMR samples and long acquisition times; Figure S8). Net interaction free energies for complexes involving the CH–π donor unit present in **C** were measured from pairwise competition experiments with equimolecular **A**/**C** mixtures. Analogous experiments with mixtures **A**/**B** and **A**/**D** did not render accurate values for the corresponding interaction energies (because of signal overlapping problems or extremely high product ratios). Therefore, the stability of complexes involving **B** and **D** were measured with respect to those formed by **C** employing pairwise competition experiments with equimolecular **B**/**C** and **D**/**C** mixtures.

Keywords: CH–π interactions · drug design · dynamic combinatorial chemistry · molecular recognition

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